The Cytoplasmic Inclusions of the Germ-Cells: Part X. The Gametogenesis of Saccocirrus.[1]

By

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With Plates 1–4 and 1 Text-figure.

Contents.

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1. Introduction.

In this paper I have attempted to shed further light on that difficult problem, the oogenesis of an Annelid: while Saccocirrus, as Goodrich has shown (26), is an Archi-annelid closely related to Protodrilus, it is probably much like other Annelida so far as its oogenesis is concerned.

I was influenced to undertake a study of the oogenesis of an Annelid by three special reasons, namely, Schaxel has described 'chromatin' emission in Aricia foetida, a Polychaete: Hempelmann and Buchner have both described most peculiar peri-nuclear extruded bodies at one period of oogenesis of Saccocirrus; and, finally, Saccocirrus is an example of precocious entry of the sperm into the young oocyte: we know that the head of the sperm lies quiescent till the oocyte is ripe, and I believe that this would offer the opportunity of observing whether the tail of the sperm entered the oocyte, and (if so) whether it took any special part in fertilization.

The behaviour of the cytoplasmic inclusions has been the object of special attention, and I have also gone into the question of the spermatogenesis, and showed in these stages the presence of peculiar yolk-spheres which are divided out between the daughter-cells at the maturation divisions. It is extraordinary the way such metaplastic bodies are shepherded into two groups during the divisions of the spermatocyte.

This is the concluding part of this present series of papers.

2. Previous Work.

The precocious entry of the spermatozoon into the young oocyte was discovered independently by three observers—F. Hempelmann, and by van Gaver and Stephan jointly. Hempelmann's paper appeared a short time before that of the two other observers.

Hempelmann has contributed two papers on this subject, one in 1906 (**34**), and one as recently as 1912 (**35**). His main conclusions are to be found in the latter paper. He shows that the sperms enter oocytes just after the end of the bouquet stage of the prophases of the heterotypic division. Only one spermatozoon enters one egg.

Just after the last stage of the prophases of the heterotypic division, Hempelmann describes peculiar granules which originate from the nucleus and pass into the cytoplasm, eventually leading to the formation of yolk. These granules are at first quite solid, grow large, and gradually become vacuolated here and there, so as to form a large number of small granules lying in a single vacuole. It is just after this has taken place that the egg cytoplasm becomes filled with yolk: speaking of these curious peri-nuclear granules, Hempelmann says, 'so scheint es mir kaum zweifelhaft, dass diese Zellbestandteile mit der Dotterbildung in Beziehung gebracht werden müssen'. With regard to the origin of the peri-nuclear granules Hempelmann writes, 'Auch der Nucleolus beteiligt sich wohl mehr oder weniger an dieser Dotterbildung, denn gelegentlich zeigt er sich umgeben von kleinen, sich ebenso wie er selbst mit den

gebräuchlichen Kernfarbstoffen intensiv färbenden Kügelchen, die wohl aus ihm hervorgegangen sind, und die an den Rand des Keimbläschens rücken, um wahrscheinlich aus diesem in das umgebende Plasma auszutreten '.

Hempelmann recognizes only one sort of plasma granule — ' yolk ', originating from the peri-nuclear ' Tröpfchen '. According to him, the entry of additional spermatozoa into the oocyte is prevented by the formation of a membrane ; the spermatogenesis has been studied by Hempelmann, who described the tripartite ' Nebenkern ' of the spermatid ; his material was not preserved carefully enough to allow of his giving a good account of the formation of the male gamete.

Van Gaver and P. Stephan have published two short notes on Saccocirrus papillocercus. In their second paper these observers state, ' Nôtre désaccord fondamental avec Hempelmann a trait à l'époque de la pénétration du spermatozoïde ; pour nous, le spermatozoïde arrive dans l'oocyte dès que celui-ci est différencié en tant qu'oocyte, lorsque sa taille est encore extrêmement minime et avant toute formation de vitellus à son intérieur. Nous n'osons pas affirmer que l'action du spermatozoïde soit la cause initiale du développement de l'oocyte, mais nous avons constamment trouvé un de ces éléments dans les ovules en voie d'accroissement et d'élaboration vitelline '. In addition, van Gaver and Stephan believe that polyspermy and assimilation of spermatozoa by the cytoplasm take place in Saccocirrus. They find ' désintégration des têtes des spermatozoïdes et, par suite, leur assimilation par l'oocyte '. The articles of van Gaver and Stephan are not illustrated by figures.

The latest observer to attack these problems was Paul Buchner (3). He showed that the whole tail of the sperm may occasionally enter an oocyte, and break up to give rise to a number of peripheral droplets, while the head of the sperm remains quiescent. The yolk-formation he describes as taking place by a partial breaking up of the nucleolus, pieces of which wander to the periphery of the nucleus—particles of yolk appearing simultaneously apparently from the nucleolar

fragments which pass through the nuclear membrane. The droplets from the sperm tail shrink up and leave the periphery of the oocyte quite clear, while the nucleolar fragments grow and multiply and fill the egg with yolk. Buchner is not altogether happy in his work on Saccocirrus oogenesis, and is very cautious with regard to yolk-formation. He ends his research with the statement, ' Die Dotterbildung von Saccocirrus verdiente wohl eine eingehendere, mit Hilfe aller zu Gebote stehenden Färbungen und Reaktionen ausgeführte Analyse '.

No observer has hitherto given any account of Golgi body or mitochondria in the oogenesis of Saccocirrus, and this form has up to the present time resisted the efforts aimed at a satisfactory interpretation of the problems which its oogenesis presents.

3. Technique and Material.

Sections of a large collection of Saccocirrus were prepared according to the plans given in my recent paper on technique (**13**). The Mann-Kopsch and Champy-Kull methods were particularly valuable. All the other techniques in current use were tried. The material used was sent to me from Plymouth, two lots in the month of June, another in July.

The worms were cut into pieces after having been killed by being dropped whole into a capsule full of fixative. Great difficulty was experienced in making successful preparations of the Golgi apparatus. All the first trials were unsuccessful, but for some unknown reason all the latter attempts succeeded completely both with Mann-Kopsch, Cajal, and Da Fano methods (**13**).

I have to thank Dr. Allen, F.R.S., of the Plymouth Biological Laboratory, for sending me the material desired by me.

Professor E. S. Goodrich, F.R.S., of Oxford, kindly placed at my disposal some of his own material of *Saccocirrus papillocercus* from Naples, and sections cut from this proved very useful.

4. Spermatogenesis.

(*a*) The Spermatogonium of Saccocirrus.

The spermatogonium is a small rounded cell with a spherical granular nucleus containing a karyosome. In Pl. 1, fig. 7, is drawn one of a group or rosette of spermatogonia: there was a spindle bridge as is common in such cases (SB). This cell was drawn from a Mann-Kopsch preparation, and the Golgi body (GA) shows as a number of black rods or batonettes surrounding an archoplasm or centrosphere. In most spermatogonia the mitochondria may be detected in the form of a cloud lying in the region of the Golgi body, as in Pl. 1, fig. 7, at M.

(*b*) The Spermatocyte.

Rosettes of spermatogonia grow synchronously to form groups of spermatocytes, and during the growth stages the mitochondria spread out through the cytoplasm, as in Pl. 1, fig. 1, M. The Golgi body grows too, and the number of its individual parts (Golgi rods, dictyosomes, or batonettes) also increases, as shown at GA. The nucleus at this period is often reniform. Possibly the most remarkable fact with regard to the spermatogenesis of Saccocirrus is the presence in many, if not all spermatocytes, of a group of true yolk-granules (lipin) quite separate from either mitochondria or Golgi body: in Pl. 1, fig. 1, the yolk-granules are at Y, and form a special cell inclusion. By the Champy-Kull method the nucleus stains bluish, the mitochondria and Golgi rods go dark red, the cytoplasm is yellowish, and the curious collection of yolk-granules stain brownish green in the osmic acid of the Champy's fluid. In Pl. 1, fig. 1, at YC on the right, is drawn one of the yolk-cells which accompanied this spermatocyte, and the yolk-granules stained the same shade as the yolk-spheres (Y) in the spermatocyte.

In Pl. 1, fig. 2, is a Kopsch preparation which shows the effect of prolonged osmication. The Golgi body (GA) has reduced the OsO_4 heavily, the mitochondria do not show, and the yolk-spherules are at Y; but besides all these there

is found a group of perfectly spherical granules at x. These went black-brown in the OsO_4: their true nature or origin was not ascertained, but it was thought that ultimately they formed a part of the spermatid, as will be shown later. The number of granules was about twelve in all the cases I could count.

(*c*) The Spermatocyte Divisions.

Pl. 1, fig. 4, is a first spermatocyte metaphase. The peculiar yolk-spherules have taken up a position at the middle of the spindle, and in the next stage (Pl. 1, fig. 5) the granules have become sorted out into two groups (y) subequal in size. Each spermatid (Pl. 2, fig. 8) receives about one-quarter of the number of granules in the spermatocyte. The behaviour of the mitochondria in the divisions is peculiar: they lose their granular state, and during the prophases break down to form threads as in Pl. 1, figs. 4 and 5, in the telophase (Pl. 1, fig. 5): the threads lie chiefly around the equatorial plate. The Golgi rods are difficult to follow through mitosis; at the prophases they lose their staining power, and it is only in certain cases that the cell at metaphase has distinguishable elements (xy in Pl. 1, fig. 4) which might be identified as Golgi elements. It must be admitted that no positive evidence has been adduced with regard to the Golgi elements during division of the Saccocirrus spermatocyte.

(*d*) The Newly-formed Spermatid.

In Pl. 2, fig. 8, is a newly-formed spermatid. The yolk-spherules (y) are on the right, while the mitochondria surround the nucleus; the Golgi elements are at ga, being scattered. In the next stage the mitochondria collect to one side of the cell, in proximity to the nucleus, the Golgi elements lying behind, as in Pl. 2, fig. 9; this cell was drawn from a Kopsch preparation, and in it no yolk-spherules could be identified. At x are what I consider to be two of the granules marked similarly in fig. 2. In nearly all Kopsch or Mann-Kopsch preparations the spermatid cytoplasm is seen to be formed of very coarse reticulum, as shown in Pl. 2, figs. 9 and 14.

(*e*) Spermateleosis.

The spermateleosis stages comprising those leading to the metamorphosis of spermatid into spermatozoon are very peculiar. The mitochondria, which in Pl. 2, fig. 9, lie grouped behind the nucleus, begin to run together as depicted in fig. 10. Three main centres for this coalescence exist, but here and there a few separate centres exist ; these ultimately join up with one of the larger centres, till one gets such a stage as in Pl. 2, fig. 11, where three balls of mitochondrial substance are produced (MM), while the remainder of the free mitochondria are gradually fusing up. By the stage of fig. 12 the mitochondria have all fused to form three solid spheres generally somewhat unequal in size, and as well, often in staining affinity. In Pl. 2, fig. 14, the three spheres are viewed from below, their unequal size being apparent.

Leaving the mitochondria at this stage, the fate of the yolk-spherules and of the Golgi elements may be described ; at such a stage as in fig. 10 of Pl. 2 the Golgi elements lie behind the zone of the mitochondria ; as the mitochondria fuse up, the Golgi elements keep behind the most distal mitochondria as in Pl. 2, fig. 11, and, finally, when all the mitochondria have fused to form the three spheres, the Golgi elements lie close up behind as in Pl. 2, fig. 12. In the case of the yolk-spherules a somewhat similar change in position has been noted : in Pl. 2, fig. 10, the yolk-spherules are on the right of the cell, but by stages in figs. 11 and 12 they have moved back behind the mitochondrial spheres.

In many, but not all, examples there can be observed between the three mitochondrial spheres, a small, often round, often angular grain, as in Pl. 2, fig. 14, at x. In figs. 9, 10, 11, and 12 such bodies are also seen.

These bodies, I believe, are derived from the grains marked x in Pl. 1, fig. 2. They seem to form a part of the mitochondrial sheath of the sperm-tail.

The spermatid at such a stage as that of fig. 12 now begins to lengthen. In Pl. 2, fig. 13, the three mitochondrial spheres

have become drawn out to form a comet-tail body attached to the spermatid nucleus. The latter has begun to undergo the usual changes which may be seen in figs. 9, 10, 11, 12, and 13. In Pl. 2, fig. 16, the tail has further elongated, the yolk-spherules and Golgi elements are drifting down the length of the tail, while the nucleus is losing its reticular arrangement. This lengthening process now goes on till the fully-formed elongate Saccocirrus spermatozoon is produced.

Owing to the small size of the cells, and possibly to the unsuitability of the material, no satisfactory description can be given of the formation of the acrosome. This can be seen in Pl. 2, fig. 13, at AS. In figs. 9 and 10, at GX, were bodies which might have some connexion with the formation of the acrosome, but of this I am unable to speak with certainty.

(*f*) The Fate of the Golgi Apparatus during Spermateleosis.

If one examines a bundle of ripe or ripening spermatozoa in material prepared by the Mann-Kopsch method, it will be found that near the end of the tail of each sperm are rounded or angular bodies which are stained by the osmic acid: in Pl. 2, fig. 17, is drawn such a bundle of sperms, the bodies being marked GAX; at a higher magnification, as in fig. 15, the bodies are seen to be very like the Golgi body drawn in the spermatogonium in Pl. 1, fig. 7, at GA. I cannot say for certain whether these bodies are derived from the original Golgi rods depicted in Pl. 2, figs. 8–13, but I should think that they are so derived, and that they have undergone some change during late spermateleosis. If these bodies are an integral part of the spermatozoon and not the degeneration products of the spermateleosis, one might expect to find some sign of them in the spermatozoa within the female: in Pl. 2, fig. 18, is section of the receptaculum seminis of a female, and drawn to the same scale as fig. 17 above. The same bodies are to be seen at the tails of the ripe spermatozoa.

5. The Fate of the Tail of the Spermatozoon during Entry.

Buchner (3) showed that the sperm-tail sometimes entered the egg, sometimes not. I have been able to confirm these observations. In Pl. 3, fig. 25, is a typical oocyte to illustrate this: the sperm-head (SP) is wrapped around the oocyte nucleus, while the remains of the tail of the sperm are, in this section, seen as four irregular chromophile bodies at SPT. In Pl. 3, fig. 22, in the lower oocyte, the sperm-head is at SP, while the tail is cut across as two irregular bodies at SPT. In the upper cell of fig. 22 the sperm-tail seems to be partly inside the egg (upper) and partly outside (lower).

While it is generally impossible to say whether these irregular masses (which we can positively identify as remains of the sperm-tail) are, or are not, inside the egg cytoplasm, when we examine eggs at a little later stage of growth, it is quite certain that in the majority of cases the sperm-tail fragments have not only entered the egg but have broken up to form a number of spherical, extremely chromophile, bodies at the periphery. In Pl. 3, figs. 24, and 23 at SPT, the beads are noted all around the periphery of the oocyte.

If Mann-Kopsch preparations be examined for this, the beads appear a pale yellow colour as in Pl. 3, figs. 19 and 21, at SPT. In some cases it certainly appeared that the number of beads derived from the remains of the tail of the sperm increased in number as the egg grew. This was probably what van Gaver and P. Stephan thought when they believed that the spermatozoa might have something to do with yolk-formation. I do not believe, however, that the beads take part in fertilization or yolk-formation, either directly or indirectly.

Later on they either disappear or become hidden by the formation of clouds of yolk or nucleolar deutoplasm (described below).

6. The Oogenesis of Saccocirrus.

The oogenesis has proved the most difficult problem that I had hitherto attacked, and at one time I despaired of ever

unravelling the intricate story of the origin and nature of the complicated granulations of the oocyte cytoplasm. After a year's work, and the making of a large number of preparations, I feel that this present account is the correct interpretation of the oogenesis. The egg cytoplasm of Saccocirrus contains four kinds of grains or formed bodies: (*a*) Golgi elements, (*b*) mitochondria, (*c*) true yolk, (*d*) nucleolar extrusions or plastin-deutoplasm.

These can all be distinguished one from another by some staining method, as described on p. 18.

(*a*) The Nucleolus during Oogenesis.

Both Hempelmann and Buchner noted the peculiar perinuclear bodies drawn in Pl. 3, fig. 23, NL, and concluded that they were in some way concerned with yolk-formation. Such a marked process as that depicted in this figure is unknown in any other animal; the history of the formation of these extraordinary attachments to the nuclear membrane is not at all easy to make out, and it is only after a study of material fixed in Champy-Kull and stained by Benda's crystal violet and alizarin that a satisfactory conclusion can be reached.

In Champy-Kull-Benda preparations the nucleolus stains a very characteristic orange-brown shade, while the mitochondria and chromatin are in shades of violet: true yolk (derived from the Golgi apparatus) is stained by the OsO_4 of the Champy's fluid. Now in such preparations the nucleolus of the young oocyte is found to be budding off small pieces, as shown in Pl. 3, figs. 24 and 25; these pieces appear to wander to the periphery of the nucleus and to pass through, but to remain plastered upon the outer surface of the membrane, as in Pl. 3, fig. 24, NL.

Some considerable variation in the exact method of this process is found: in certain cases the pieces broken from the nucleolus are coarse and easily distinguishable, as in Pl. 3, fig. 24, but in some other examples, of which fig. 25 is hardly typical, the broken-off pieces are so small that they are difficult to identify.

Occasionally, as in Pl. 3, fig. 22, the nucleolus may be seen to be differentiated at its periphery into a number of small stainable bodies which may represent the beginnings of the parts to be extruded.

In Pl. 3, fig. 23, is a cell showing the appearance of the nucleolar extrusions after staining in iron haematoxylin or Champy-Kull, while in fig. 21 is a cell treated by Mann-Kopsch and the nucleolar extrusions appear as pale yellowish bodies.

From the stages represented by Pl. 3, figs. 21 or 23 onwards, there is generally marked difficulty in ascertaining the exact fate of the nucleolar extrusions. This is due to the fact that just about this period a second process is set into motion; this consists of an appearance all around the nuclear membrane of a chromophil cloud, which in most preparations obscures the nucleolar extrusions; an exaggerated example of this is drawn in Pl. 4, fig. 35, from a silver nitrate Da Fano preparation, but the cloud does not show so darkly with Benda or iron alum haematoxylin.

At all events there begins at this period a peri-nuclear activity, which also corresponds with the change of the chromophility of the egg cytoplasm from a primary oxyphilia to a basophilia. Two other occurrences also tend to obscure the peri-nuclear nucleolar bodies at this period: around each body a clearly-defined vacuole often appears (Pl. 4, fig. 29, NLV, from a Mann-Kopsch preparation), and moreover the mitochondria near the nuclear membrane are now forming actively-growing and dividing clusters. With iron alum haematoxylin it is not possible to make sure as to the fate of the nucleolar extrusions, because these and the mitochondria stain in the same colour. With the Champy-Kull fixation and Benda stain I have found examples which, I believe, establish as a fact my view that the nucleolar extrusions first lose their connexion with the nuclear membrane and then either pass right away into the cytoplasm or immediately begin to break up into much smaller fragments. As with the mode of appearance itself of the nucleolar extrusions, so also the subsequent behaviour of these bodies is open to a good deal of variation.

With almost a suddenness a large number of nucleolar yolk-bodies appear in a ring surrounding, but some distance away from, the nucleus (Pl. 4, fig. 34, is a somewhat later stage).

The appearance of this ring of numerous nucleolar yolk-bodies corresponds more or less closely with the spreading out of the basophil peri-nuclear cloud referred to below (p. 17).

The next period sees the complete change of the cell from primary oxyphilia to a temporary basophilia: this activity is often shown plainly with the Mann-Kopsch osmium tetroxide method, of which Pl. 4, fig. 29, is an example; the whole appearance of the cell seems to change. Later the peri-nuclear vacuoles disappear, the cytoplasm becomes smooth, and the nucleolar yolk-bodies are the most noticeable element in the egg.

In Pl. 4, fig. 30, is an egg fixed for six weeks in formol-Flemming; the true yolk (derived I believe from the Golgi elements) has gone black with the osmic acid, while the nucleolar yolk-spheres are pale, in this case fuchsinophile. bodies; neither mitochondria nor Golgi elements appear in this preparation.

That the nucleolar deutoplasm or yolk-spheres go on dividing in the egg cytoplasm seems to me a very likely suggestion, but I was unable to prove that such was the case. How then otherwise can we account for the extraordinarily rapid increase of clouds of these alizarin-staining granules such as appear in Pl. 4, fig. 34? It seems certain that smaller nucleoli inside the nucleolus keep budding off extra-nuclear fragments (Pl. 4, fig. 34), but this would not account for the arrangement and rapid growth of clouds of granules such as those at NL in Pl. 4, fig. 34.

In Pl. 4, fig. 30, which was drawn from a very clear example where the nucleolar yolk-spheres were large, I could not see any of the latter undergoing binary fission; I am therefore disposed to believe that these cytoplasmic nucleoli bud off little pieces just as the larger nucleolus is doing in the cell drawn in Pl. 4, fig. 34; and then these little pieces themselves grow larger.

In Pl. 4, fig. 31, is a part of the cytoplasm of a nearly ripe

oocyte ; it will be noted that there are now enormous numbers of granules formed, and the majority of these are nucleolar deutoplasm derived from the original nucleolus of the oogonium.

(*b*) The Mitochondria.

In the young oogonia I did not find it possible to demonstrate mitochondria, but in all the oocytes just at or after the last stages of the prophases of the heterotypic division, mitochondria are easily identified, especially after proper staining in iron alum haematoxylin. In Pl. 3, fig. 22, are two oocytes showing the fine grey-staining bodies which I have identified as mitochondria. These show more clearly in Pl. 3, figs. 24 and 25 ; the mitochondria do not appear to have anything to do with the nucleolus.

(*c*) The Golgi Apparatus.

The Golgi apparatus (Golgi body or element) was studied by the Cajal, the Da Fano, and the Mann-Kopsch techniques ; of these the Mann-Kopsch technique was the most suitable. In young oocytes the Golgi apparatus consists of an excentric juxta-nuclear mass, as at GA in Pl. 3. fig. 20. This mass really lies around an archoplasm, as in Pl. 3, fig. 24, at AR. In Pl. 4, fig. 33, is an oocyte showing the Golgi apparatus on the right of the nucleus. Now in the youngest oogonia the Golgi body is isolated at one side of the cell, but quite early in the history of the progerminative oogonium it grows rapidly and begins to fragment ; the additional pieces so derived move out into the other regions of the cytoplasm, as has already happened in all the three cells drawn in fig. 20 of Pl. 3. In fig. 33 on the same plate (though the oocyte is drawn much older in so far as the extrusion of nucleolar deutoplasm is concerned) the Golgi body is still fairly isolated, being just in process of fragmentation. In some cases, as pieces break off from the original body, they pass away into the free parts of the cytoplasm and form remarkable nests or areas of proliferation, as in the cell in Pl. 4, fig. 28, at GA. While in certain cases the fragments of Golgi body scarcely retain their semi-lunar

spherical condition, in other examples this condition is retained, as in Pl. 3, fig. 19, which was a remarkably clear example ; the neighbouring cell in fig. 21 showed this condition less well, while in fig. 33, most of the smaller fragments were of no special shape. In Pl. 4, figs. 28 and 29, the Golgi elements form a fine dust at the periphery of the cell. In all the cases, however, the ultimate result is the same—the apparatus breaks up into hundreds of irregular grains, as in Pl. 4, fig. 32, at GA.

If the Saccocirrus is prepared by the Mann-Kopsch method, and the sections mounted in balsam without any previous treatment, the cell-granules of the oocyte appear as in Pl. 4, fig. 31 ; here we find a confused mass of granules which have become either blackened or browned to different degrees. But if the sections on the slide be treated for several hours in turpentine all but the Golgi granules become decolourized or a light yellow in colour. In Pl. 4, fig. 33, the egg-granules were throughout the colour of those in fig. 31, but the slide was treated in turpentine and the colour extracted from everything except the apparatus, which is here seen to be fragmenting and spreading through the cytoplasm.

(*d*) The Formation of Fatty Yolk.

In the egg of Saccocirrus which has been centrifuged, a layer of fatty yolk of an oily type collects on the upper pole of the egg (see Text-fig. 1). The characteristics of this yolk are that it goes greenish or brown only after prolonged osmication and is rapidly destroyed by fixatives containing lipoid solvents.

Such fatty yolk is quite distinct from both nucleolar deutoplasm and mitochondria, but it is best shown by Kopsch techniques which demonstrate the Golgi elements so well. In Pl. 4, fig. 30, the fatty yolk is shown black and the nucleolar deutoplasm yellowish grey, after prolonged immersion in formol-Flemming.

From the method and time of appearance of the fatty yolk I believe it is formed from the Golgi bodies, but I admit it is impossible to make a trustworthy statement in such unfavourable material.

(*e*) Changes in Chromophility during Oogenesis.

Method I.—Fixation in saturated solution of corrosive sublimate, staining in Ehrlich's haematoxylin and eosin according to Scott's directions (**55**). The nucleolus of the oogonium is amphophil with distinct basophil preponderance, i. e. more blue than reddish purple. The chromatinic reticulum becomes oxyphil after a certain time, and remains so throughout oogenesis ; the oogonial cytoplasm is oxyphil. During oogenesis at the period of the appearance of the peri-nuclear bodies (nucleoli, Pl. 3, fig. 23) the cytoplasm becomes basophil, especially near the nucleus. This basophility persists in a peri-nuclear position for a considerable time and spreads out, but gradually the entire cytoplasm again becomes completely oxyphil.

A typical somatic cell (e.g. gut, or epidermal) shows an oxyphil cytoplasm and basophil nucleus. The head of the sperm is basophil, the tail oxyphil.

Method II.—The same material stained by eosin and toluidin blue (in this order) offered a new point of view. Somatic nuclei were blue, the sperm-head and most of the epidermis cytoplasm blue also. The body-muscles and the tail of the sperms were red. The oocyte cytoplasm had an oxyphil ground, but the nucleolar deutoplasm was bluish. The nucleolus itself was generally amphophil, and, as in the case of the Ehrlich preparation, had a basophil central core and an amphophil cortex with basophil preponderance. In other cases the nucleolus was entirely oxyphil. The peri-nuclear bodies were completely blue.

While these results are in themselves of little importance from the point of view of the detection of peri-nuclear ' chromatin omissions ', they show very clearly that at the time when the primary oxyphilia is changing to the basophilia there is great new activity in the region of the nucleus ; this activity leads to the formation of new denser cytoplasm, and it will be noted below that there is a correspondence between the pictures given by methods explained above and with formalin silver-nitrate or chrome-osmium techniques.

(*f*) On Peri-nuclear Activity.

In Pl. 3, fig. 25, is drawn a young oocyte at a time when the nucleolar deutoplasm is being formed ; this cell was prepared by Da Fano's cobalt-nitrate-formol-silver-nitrate method. It is remarkable for the fact that it demonstrates very clearly the extraordinary peri-nuclear activity at this stage of oogenesis. It is possibly this material which stains basophil as described on p. 16. In all my silver-nitrate preparations the oocyte at this stage shows this peculiar appearance.

The peri-nuclear cloud stains black or grey according as to whether the preparation has or has not been toned, while the nuclear reticulum and nucleolus are only faintly yellowish and may subsequently be stained bright red in safranin.

I look upon this cloud as the direct result of active protein metabolism around the nucleus ; the protein is possibly forming under stimuli sent forth from the nucleus. There is no evidence that this cloud is chromatinic, for the Golgi silver-nitrate methods (Golgi, Cajal, Da Fano) do not impregnate chromatin in any cells I have studied. There is, in addition, no evidence of intra-nuclear specks or dust as described by Schaxel for Aricia foetida.

Later on this cloud disperses through the whole egg cytoplasm.

7. Some Chromophility Reactions of the Four Categories of Cytoplasmic Granules.

The table given on p. 18 summarizes the differences which can be shown to exist between the four categories of cytoplasmic granules found in the egg of Saccocirrus. Only those methods which best show these differences are mentioned, but besides I used many other fixing and staining techniques (13).

The nucleolar yolk-spheres or deutoplasmic elements approach somewhat in their density to the mitochondria and tend to stain rather like them. Besides these methods quoted there are such tests as the use of alcoholic or acidified

(acetic) fixatives, which either wash away the fatty yolk or the mitochondria, or both, and leave the nucleolar deutoplasm. Then there are the formalin silver-nitrate methods which stain the Golgi elements. The table below shows the following:

Method 1 constitutes a difference between mitochondria and nucleolus and its derivatives.

Method 3 constitutes a difference between nucleolus and its derivatives and true fatty yolk (from Golgi elements).

Method 4 constitutes a difference between nucleolus and Golgi apparatus.

Method 5 constitutes a difference between Golgi apparatus and mitochondria, as also do Methods 1, 2, and 3.

TABLE.

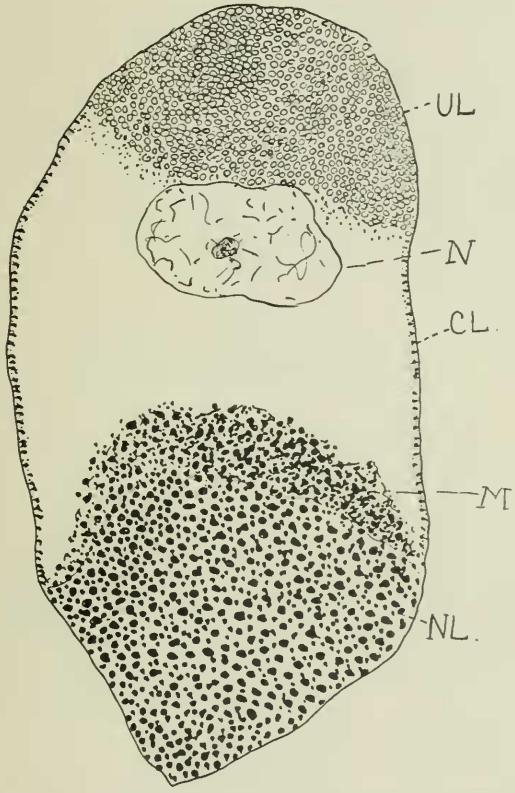
Method Used.	*Nucleolus and its Derivatives.*	*Golgi Apparatus.*	*Yolk.*	*Mitochondria.*	
Champy - Kull fixation. Benda's alizarin and crystal violet	Yellow-brown	Does not show	Black	Violet	1
Flemming without acetic acid, and iron haematoxylin	Black	Does not show	Greenish-brown	Black or grey	2
Formol-Flemming and Altmann's acid fuchsin	Reddish	Does not show	Black	Did not show plainly	3
Mann-Kopsch	Yellowish	Black, difficult to decolourize in turpentine	Black, easy to decolourize in turpentine	Did not show	4
Mann-Kopsch, Altmann	Reddish	Black, and as above	Black, and as above	Red	5

8. Centrifuge Experiment on the Ovarian Oocyte.

Live specimens of Saccocirrus were placed in a tube and centrifuged for twenty minutes at 3,000 to 5,000 revolutions a minute. They were afterwards thrown into capsules of fixatives of various types. The centrifuged egg shows three layers, viz. an upper cap, a clear subcentral zone, and

a large lower zone. The upper cap is formed of delicate granules which I think are fatty yolk and probably of the Golgi elements ; these granules will go yellowish green

TEXT-FIG. 1.



Centrifuged oocyte of Saccocirrus. Shows an upper layer of greenish oily yolk (UL), clear middle layer with nucleus (N), lower layer principally nucleolar deutoplasm (NL) with an upper layer mainly mitochondrial (M). At CL are the cortical lamellae of the egg membrane. (Chrome-osmium and Benda stain.)

after prolonged osmication. The middle layer generally shows two zones—an upper just beneath the fatty yolk and staining in crystal violet, or iron haematoxylin, and looking much like thickened cytoplasmic reticula ; then there is the

large lower area formed of the heavy nucleolar deutoplasm, forming by far the largest separate part of the centrifuged oocyte.

These areas are shown in Text-fig. 1. The nucleus generally lies in the middle layer. The mitochondria appear to be lighter than the nucleolar deutoplasm and take up a position in an area above the latter, M in Text-fig. 1, but are also found throughout the lower area.

Around the exposed periphery of the egg, the cortical lamellae are beautifully apparent, especially in Benda preparations (CL in Text-fig. 1). It is from these lamellae that the substance of the fertilization membrane is produced. See also lamellae in Pl. 4, fig. 27, CL.

9. Cells Found in the Male, intermediate between Spermatocyte and Oocyte.

In the coelom of the male Saccocirrus are found large cells packed with yolk-spheres; these large cells often fill up all the coelomic space in the mid region of the body, excepting for the areas occupied by developing spermatozoa.

Occasionally one finds large isolated cells lying completely surrounded by and shut in between the large yolk-cells. These isolated cells were once young spermatocytes, which, during growth of the yolk, have become shut off. That this is so is indicated by an examination of a sufficient number of Saccocirrus males.

Now these isolated cells are sometimes remarkable for the fact that they show a rough resemblance to oocytes at the stage of nucleolar extrusion. In Pl. 1, fig. 3, is drawn such a cell. The group of yolk-granules is at Y, several groups of fine mitochondria are at M, M, while the nucleus is found to be in the process of extruding large peculiar nucleoli, NLX. In this one section the nucleus showed four pieces being extruded, two other nucleoli inside the nucleus, and one piece on the lower right of the cell already detached from the nucleus; an examination of the larger nucleoli in fig. 3 shows that they

have a stout triangular base quite like the bodies in Pl. 3, fig. 23.

When I came to examine my first preparations illustrating the oogenesis of Saccocirrus, I did not immediately notice the fine mitochondria drawn in Pl. 3, figs. 22 and 23, and I was temporarily led to believe that the nucleolar extrusions might represent the mitochondria. But before I had made more and better preparations, as the result of my experience on this new material, my belief in this view that the nucleoli might represent mitochondria was shaken by finding such cells as that in Pl. 1, fig. 3, in which I noted both mitochondria and bodies which, I concluded, represented the extruded nucleoli of the oocyte.

With regard to the probable reason for the appearance of nucleolar extrusions in a spermatocyte, I believe that it is due to the fact that such cells are packed away among yolk-cells which bring about conditions simulating the metabolism of the egg-cell.

10. Discussion.

(*a*) General.

The oogenesis of Saccocirrus is likely to be typical of several other Annelida, and possibly of Polychaeta such as Chaetopterus and Nereis, judging from Lillie's figures of centrifuged ova of these genera.

A graphic representation of the oogenesis of Saccocirrus would be as follows :

Oogonium.	Full-grown Oocyte.
1. Nucleolus .	1. Nucleolus. 2. Nucleolar deutoplasm or nucleolar yolk-spheres.
2. Golgi elements .	3. Definitive Golgi elements. 4. Yolk-spheres (fatty).
3. Mitochondria .	5. Mitochondria.
4. Chromosomes .	6. Chromosomes only.

The part of this scheme about which I feel some doubt is the metamorphosis of the Golgi element into a yolk-sphere :

that such takes place in Ascidians and Molluscs is now quite certain, but the egg of Saccocirrus does not provide such clear opportunity for study as that of Limnaea or Ascidians. Nevertheless, I believe that I have made sufficiently clear observations on a large amount of material to justify the above interpretations. It is only after the dispersal and breaking up of the Golgi body that true fatty yolk puts in an appearance, and in many cases it seems that Golgi elements can be traced step by step as the eggs grow, metamorphosing into fatty yolk. The extruded nucleolar material has nothing to do with this, and I do not think that the mitochondria are concerned in the process.

When we take the case of extruded nucleolar material in Saccocirrus it is difficult to understand why the bulk of the formed reserve granules in the egg should be of nucleolar origin. If one studies the cytoplasm of the egg in a number of different examples of oogenesis, one sometimes finds that the nucleolus supplies the bulk of reserve material (Saccocirrus), sometimes the Golgi apparatus (Patella or Limnaea), sometimes the mitochondria, as in certain insects. In each of these cases, however, analysis of the entire reserve materials in the egg cytoplasm leads one to a similar conclusion for each example, namely, that reserve material in eggs of invertebrates consists of protein and fat or lipin (or both these). Then, of course, most eggs contain glycogen.

In some examples, as in the sponge Grantia, the main bulk of reserve material seems to be delicate vacuoles of lipin originating from the ground plasma.

(*b*) The Preciseness of the Modern Technique for the Cytoplasmic Inclusions and for Chromatin.

Some observers apparently unacquainted with the finer usages of modern cytological technique have written doubtfully of the preciseness of such methods. In all the cytological problems that I have attacked the difficulty I have met with lies not especially in the discrimination between yolk, Golgi elements, mitochondria, nucleolar deutoplasm, and

glycogen, for this was generally easy, but in the identification of chromatin; the problem was whether basophile chromatic material was chromatinic; this is the great problem of cytology at the present time.

I believe that I may be forgiven for holding an optimistic view with reference to our present and future understanding as to the behaviour of the Golgi elements and mitochondria during oogenesis: I think that the works of Jan Hirschler, Weigl, Nussbaum-Hilarowitz, Rio-Hortega, and my own series of papers on the cytoplasmic inclusions have gone far to shed a clear light on the subject, but I do at present feel much puzzled over the nuclear phenomenon in oogenesis.[1]

One is driven onward trying to avoid the pool of Charybdis, formed by the chromosome theorists who will not admit of true chromatinic extra-nuclear extrusions, and the rock of Scylla, which in my mind is constituted by the fact that it is at times difficult to believe that the so-called extra-nuclear extrusions are not chromatin. This special matter is further discussed below under the heading of ' The Supposed Chromatinic Nature of Extruded Nucleolar Material '.

In all probability were it not for the ingenious, and one must say believable theories of chromosome workers of Morgan's, Wilson's, or McClung's schools, one would have no hesitation in saying that the extra-nuclear extrusions were chromatin, even though they frequently do not stain quite like the chromatin of the ' resting ' nucleus. When one takes the case of the secondary nuclei of the Hymenopterous egg it is very difficult to avoid the conclusion that such granules are chromatin.

This is a matter to which I have given a good deal of attention. Quite recently I have again gone over my Apanteles material, and I have found an example which shows the chromatin filaments at the diplotene stage of the prophases of the heterotypic division, while the nucleolus is separate and shows buds, some of which have already passed into the cytoplasm to form minute secondary nuclei.

[1] Mr. R. J. Ludford's recent work has gone far to clear up parts of this obscure ground (' Jour. Roy. Micr. Soc.', 1920–21).

While this helps towards a disposal of the view that the chromosomes, at this period at least, are drawn upon to provide material for the formation of secondary nuclei, it does not dispose of the questions as to the nature and origin of the nucleolus which buds off the secondary nuclei.

It is possible to recognize several kinds of nucleolar activity in various examples of oogenesis.

Saccocirrus nucleolus .	definite nucleolus. nucleolar deutoplasm.
Apanteles nucleolus . .	definite nucleolus. secondary nuclei (associated with yolk-formation).
Grantia nucleolus . .	definite nucleolus. mitochondria (' chromidia ').

In certain other forms it is possible to recognize a process of nucleolar extrusion early in oogenesis, but which appears to lead to nothing (possibly in Patella).

The belief held by some observers that nucleolar extrusion may be looked upon as a process whereby the nucleus sends chemical messengers into the cytoplasm inducing growth to begin, is discountenanced, for Saccocirrus at least, by the very apparent fact that nucleolar extrusion is prone to much variation in the point of time and the rate that it takes place—as shown by comparing the sizes of the eggs in Pl. 3, fig. 23, and Pl. 4, figs. 29 and 33. The process is just beginning in the first-mentioned figure, and has finished in the smaller egg in the last-mentioned figure.

(*c*) On the Supposed Chromatinic Nature of Extruded Nucleolar Material.

If one fixes the testis or ovary of any animal in Zenker or Petrunkewitsch fluid, and stains in Ehrlich's or Delafield's haematoxylin and eosin or Biebrich's scarlet, it will be noted that during the greater part of the development of the sperm the chromatin stains blue, or basophil ; but there are certain periods when what we can only assume to be true chromatin,

as it is morphologically derived from preceding materials which stained like chromatin, will be found to stain oxyphil, or in the red stain. As Bayliss especially has shown clearly in his valuable 'Principles of Physiology', staining depends on a number of more or less obscure factors, and it is probably injudicious to lay too much weight on the results of staining fixed material. In many of the parasitic Hymenoptera the egg nucleus contains a large heavily-staining nucleolus which buds off fragments, which pass through the nuclear membrane into the egg cytoplasm, where they form what are known as secondary nuclei. With safranin and light green the nucleolus of the true egg nucleus stains red, and the nuclear (chromatinic) network a green colour. In the sponge Grantia the plasmosome of the oocyte partly passes into the egg cytoplasm to form bodies called by Jörgensen and Dendy 'chromidia'; I have objected to the use of this term for such nucleolar fragments, both because we do not know that they are chromatinic and also because such 'nucleolar' extrusions appear to be identical with the mitochondria.

We must face the facts frankly: the chromosome theorists would object to the identification of 'nucleolar' extrusions as chromatinic in nature and as derived from the definitive chromosomes. I have shown above that staining tests are not conclusive; several others, and also I myself, have demonstrated that the secondary nuclei are derived from extruded fragments which in the case of such forms as Myrmecina or Apanteles are, I believe, to be regarded as of nucleolar origin. We find, therefore, that fragments of the nucleolus can form a true nucleus, with nuclear membrane, linin network, and nucleolus.

Seiler (**55** *a*) described in Lepidopterous eggs what he has called a chromatin diminution process; the polar body spindle at metaphase is found to carry three groups of granules, the two outer being the chromosomes which have divided and are becoming separated, the middle group of granules being apparently derived from the ends of the chromosomes by a diminution process, well known in the somatic mitoses of

the developing Miastor egg (32). Just before his untimely death Professor L. Doncaster was examining this problem, and sent me some of his slides for examination and suggestions; all that I could do was to recommend the use of stains such as Auerbach and Pappenheim, and methyl blue eosin. Digestion tests and such other microchemical tests are impossible when one is working on the minute spindle in a very small egg. It certainly seemed to me that in the slides sent by Professor Doncaster the intermediate bodies were derived from the ends of the chromosomes as in Miastor.[1] Here again, however, we are faced with the same difficulty with regard to staining test, as I have pointed out with reference to the nucleolus: we are not justified in saying that a substance is chromatin simply because it selects methyl green from the Pappenheim or Auerbach stains; no one would care to say that the head of the spermatid was not chromatin, yet at certain periods it will select the red stain from the Pappenheim or Auerbach fluid. To my mind it is useless to declare that the head of the sperm at such stages is not true chromatin, but has only changed its chemical nature; the head of the sperm is derived from chromosomes before it reaches the egg and breaks up into chromosomes when it has penetrated into the egg. The spermatid nucleus takes the red stain from the Pappenheim or Auerbach fluid possibly because the arrangement of its surface or internal substance is more favourable to the molecules of the red stain, and unsuited for the absorption of the green stain.

The facts of the matter are that we know very little about the relationship between the nucleolus and the chromosomes, both during mitosis and during interkinesis; the same remark applies when we come to the subject of the microchemical nature of the nucleolus. I believe that a good step towards the elucidation of the first-mentioned problem has been taken by H. M. Carleton.

This observer has shown that the nucleolus of certain vertebrates contains an argentophil core, or is related more

[1] I have often wondered why this work of Prof. Doncaster was not edited and published.

or less closely to a body which under certain conditions becomes densely black in Cajal's formalin silver-nitrate technique for the Golgi apparatus. During mitosis Carleton has shown that the argentophil core which he calls a nucleolinus (Haeckel) does not lose its individuality but divides, and may be found among the two chromosome groups of the telophase. I have been enabled to go through the preparations made by Carleton and can vouch for the correctness of his description; moreover, I possess preparations of the gut-cells of Saccocirrus, of the follicle-cells of Stenobothrus, and of many tissues in Rana, all of which show a typical nucleolinus. What is very important is that Carleton has shown that the nucleolinus may be associated with either a 'karyosome' or a 'plasmosome' type of nucleolus. These remarks will serve to indicate the importance of work carried out on the nucleolinus, especially with Cajal's formalin silver-nitrate method; Da Fano's cobalt-nitrate method also serves to bring out the nucleolinus in some forms.

Interpreting the work of Carleton on the nucleolinus, and also in the light of Cajal's figures of various mammalian tissues and my own materials of invertebrates, I believe that the nucleolus, term used generally, might be morphologically independent of the chromosomes during the germ-cell cycle; the nucleolus during interkinesis might exist as a compound body consisting of a core which is argentophile and sometimes chromophile to other stains, and this core might act as the centre for the proliferation of a more extensive body which functions as the plasmosome or karyosome of the 'resting' nucleus; furthermore, during mitosis this outer region proliferated from the argentophil core possibly becomes lost, to be reformed in the next interkinesis. How far these suggestions will be found correct is impossible to say at present, but many of the facts we know now point in the direction I have indicated. Moreover, this view would coincide with the already-formed theories of the chromosome worker.

The nature of the nucleolus is mainly proteid, maybe even in some cases nucleo-proteid, but its functions appear to be different from those of the chromosomes. The nucleolus, like

the chromosome, Golgi element, and mitochondrium, is capable of growth and binary or multiple fission.

Buchner, in his paper on the secondary nuclei of parasitic Hymenoptera, among the other conclusions, comes to the two following: accessory nuclei are to be traced back at the beginning, as naked chromatin (sic) granules lying in the cytoplasm. From these granules develop enchylema, nuclear membrane, and linin network, while the granule itself becomes the nucleolus of the accessory nucleus. Buchner has used safranin and light green and iron haematoxylin as stains; he labours under the delusion that what stains in a basic dye must necessarily be chromatin. He states that the chromatin granule which induces the formation of karyolymph, linin network, and nuclear membrane, later becomes a 'nucleolus'. Buchner figures the oocyte of Bombus and Myrmecina showing the nucleoli of the head nucleus as red granules (safranin) and a more or less faint chromatin (?) network green ('lichtgrün'). The accessory nucleus also shows a red nucleolus and a green network. Buchner and others have concluded that the red-staining substance of the head nucleus, which becomes extruded through the nuclear membrane, is chromatin. As I have mentioned before I do not believe that one should lay too much weight on the staining tests (and Buchner has not tried several of the stains I should like to have seen used), but the points which must be emphasized are, firstly, that it is proven that the nucleolus of many hymenopterous insects does fragment and partly pass into the cytoplasm; and secondly, that these fragments do form secondary nuclei, exactly similar in certain species, to the head or principal nucleus. Call the red-staining body inside the head nucleus what one may, plastin or chromatin, plasmosome or karyosome, it is a fact that fragments of it can give rise to secondary nuclei.

There is some temptation to use the facts which have recently been described in parasitic Hymenoptera, and in this paper, with reference to the behaviour of nucleoli, as support for a 'binuclearity' hypothesis of some kind. In a recent paper

on the giant germ nurse-cells of Testacella (**4**) I ventured to interpret certain of my results in this manner, and it must be said that the case of the secondary nuclei is very suggestive.

There are three possible modes of general interpretation—either the nucleolus represents a second chromatin of some kind, but separate from the chromosomes, or it derives its chromatin from the chromosomes, or there is some cell substance other than chromatin which has the attribute of forming bodies similar to the ordinary nuclei, except for the presence in them of true chromatin. Whether the power of production of a nucleus-like body is to be looked upon as a proof of the chromatinic nature of a granule is unknown.

(*d*) On the Special Part played by the Nucleus during Oogenesis.

Recent studies on the cytoplasmic inclusions of the germ-cells have revealed the fact that all such units possess both Golgi elements and mitochondria, and that these two categories of formed elements take a prominent part in the upbuilding of the egg cytoplasm. No one has claimed a nuclear origin for the Golgi body, and in my work I have found a complete Golgi apparatus in the earliest germ-cells which have been studied—in molluscs, insects, birds, amphibians, and mammals. The case of the mitochondria is different; several observers have claimed that they have found the mitochondria to originate from the nucleus during early stages of oogenesis or spermatogenesis. I had never seriously believed these accounts, and still doubt most of them: but in my own studies on the sponge Grantia I was led to identify the 'chromidia' of Jörgensen as the representatives of the mitochondria; now Dendy firstly, and then I, have shown that the 'chromidia' of Jörgensen are nucleolar in origin. I still have some doubts as to whether true mitochondria do not exist in Grantia, but my efforts to demonstrate other granules which might be mitochondrial have so far not met with success; therefore I can but assume tentatively that in the case of Grantia the mitochondria are of nuclear origin.

It is important to notice that careful modern work on oogenesis confirms certain previous accounts of the extrusion of nucleolar material into the egg cytoplasm, and puts on a definite basis of truth the claim that the nucleus takes a part in the development of the cytoplasm.

All such positive evidence which we possess in this direction applies to the behaviour of the nucleolus, and I do not believe that we are able to point to any circumstances which would lead us to conclude that the chromosomes take a part, though I think that such is the case. Probably the only significant fact upon which we can fall back lies in the formation of flocculent threads and reticula from the chromosomes after the prophases of the heterotypic division, and just before the real inception of the growth period of oogenesis. But this might just as well be interpreted as preparation by the chromosomes for their own growth by means of substances absorbed from the egg cytoplasm.

With regard to this difficult matter of the relationship between nucleus and cytoplasm during oogenesis, I believe that zoologists may be able to ascertain new facts if they develop and use more constantly the various silver-nitrate techniques, which give pictures unobtainable by other methods.

(*e*) Schaxel's Chromatin Emission.

From time to time in these papers I have referred to Schaxel's work on chromatin emission in a number of invertebrates which he has studied. Criticisms which have already been brought forward by me, in conjunction with Woodger, are that Schaxel has not worked at his material by proper methods, and he has not attacked the problem from the point of view of the cytoplasmic inclusions. Furthermore, he has not established that his granules are chromatin or that they are emitted through the nuclear membrane. With corrosive fixation, &c., and Ehrlich's haematoxylin, the granules are found to be basophil, which probably proves nothing with regard to their microchemical nature. A new phase in the problem of Schaxel's work was introduced by Miss van Herwerden, who, by treating

Strongyloentrotus eggs in a 'nuclease' procured from spleen and pancreas, succeeded in dissolving away Schaxel's granules, which did not appear when the eggs were subsequently treated by methods which fixed and stained the granules in eggs not treated by the enzyme solution.

This work has been especially referred to by some recent writers, who consider that weight should be attached to Miss van Herwerden's statements.

With certain precautions, which were incomplete, she prepared a proteolytic enzyme from spleen, according to the directions of Sachs (**52**). Now I submit that her enzyme solution was probably a mixture of several enzymes, 'nuclease' possibly, but also lipolytic enzyme as well. The fact that cell granules disappear under treatment by such a solution proves nothing with reference to their precise chemical nature. These granules were possibly mitochondria whose proteid basis was washed away by some protease, which would cause them to disappear as definite granules—or what is more likely, Miss van Herwerden's 'nuclease' contained a lipoclastic body which swept away the linin content of the mitochondria.

Until an expert on enzymes prepares solutions whose contents are known and whose reactions towards various organic materials are completely worked out *in vitro*, until the microchemistry and origin of bodies in question are better understood, then and then only should one place any weight on such work by enzyme action as that of Miss van Herwerden on Schaxel's 'chromatin' granules. It should be noted carefully that Schaxel's granules do not produce bodies resembling nuclei, as happens in Apanteles, &c.; one should not without good reasons call any haematinophilous body chromatin: even if his granules are extruded from the nucleus, they might just as well be nucleolar as chromatinic; and he might with advantage try other methods.

Zoologists should note carefully that an espousal of Schaxel's views seems to necessitate either the further adoption of a binuclearity hypothesis or the rejection of the chromosome theory.

For if Schaxel's granules are chromatin, using the word in the sense that they are made of the same sort of material as the chromosomes, either they must have originated from the latter—have been budded off from them—or there must be two kinds of chromatin in the egg nucleus.

I cannot see how the adoption of the first alternative will allow one still to hold that the present-day chromosome theory is likely to be true ; and the very behaviour of the nucleolus in Apanteles shows that there is a body other than the chromosomes which can produce a nucleus.

By placing one's belief in the second of the two alternatives—in some form of ' binuclearity hypothesis ', one could also make many of Schaxel's observations fit in with the more theoretical aspect of the question.

While my mind is as open as it well could be in view of my own observations, I do not at present feel that Schaxel has attacked the problem in the best way, and I refrain from definitely accepting any of his views till some other observer carefully reinvestigates his claims and uses all the best and latest cytological techniques.

Perhaps it should be mentioned that the above remarks do not commit me to the espousal of any ' binuclearity hypothesis ', though I feel that there is some good evidence for such a postulation.

(*f*) Centrifuge Experiments in Annelid Development and what they demonstrate.

It has been shown in this paper that the major part of the granules of the egg of Saccocirrus is derived from nucleolar material extruded from the nucleus. If these nucleolar extrusions represent Schaxel's chromidia or the granules which form the secondary nuclei in parasitic Hymenoptera, and if they are of chromatinic nature, and not merely metaplasm or yolk, one might expect them to play some special part during embryonic development. They might even represent organ-forming materials.

But apparently this is not the case : Lillie (43) has given

some figures of centrifuged eggs of Chaetopterus and Nereis which lead me to believe that in these animals the egg contains fatty yolk (or oil) and nucleolar deutoplasm as in Saccocirrus. In Chaetopterus he finds the layers in the centrifuged egg to be a grey cap, upper (the ' fatty yolk ' of this paper), a clear area in the middle, and a lower layer of ' yolk ' (my ' nucleolar deutoplasm ' and mitochondria) ; these areas correspond with the layers in the centrifuged Saccocirrus egg (p. 18).

Now, speaking of these layers in developing embryos and of formative stuffs in general, Lillie remarks: 'So far as they (formative stuffs) are to be identified with the visible substances segregated by the centrifuge, it would appear to be indicated by experiments that they can play no specific rôle in differentiation, because in centrifuged eggs they may occupy variable positions in the embryo.' This view coincides with that of Morgan (quoted in my previous paper (17)) and with Miss Beckwith's study on Hydractinia. Any physiological derangement during the development of centrifuged eggs seems to be due either to mechanical difficulties of massed yolk or to absence of nutriment.

It is interesting to note, too, that Morgan came to his conclusion partly as a result of work on Echinoderm eggs, where Schaxel finds an emission of ' chromatin ' granules.

(*g*) The Probable Part played by Mitochondria and Golgi Apparatus in Heredity.

Modern cytologists tend to become divided into two groups—those working on the nucleus and those working on the cytoplasm. Nearly all modern text-books dealing with Heredity and Sex treat exclusively of the part played by the chromosomes in the mechanism of Heredity, and most observers are satisfied to accept the view that ultimately the nucleus is the seat of the substances which contribute to bring about the phenomena of Heredity. ' Die Mitochondrien sind die protoplasmische Vererbungssubstanz ' is a statement which serves to show us that the chromosome theorist is not alone in this field. In the germ-cell cycle the chromosomes have been

shown by Van Beneden, Boveri, Wilson, Morgan, Montgomery, McClung, Doncaster, and many others, to go through certain definite changes, which have been found to correspond with many of the peculiar phenomena of sex and heredity in breeding experiments. The main facts ascertained with regard to the chromosomes are briefly as follows:

1. They are constant in number in any one species.
2. In ordinary cell-division each chromosome is halved so that each moiety is a complete replica of its fellow.
3. In the formation of the germ-cells there is a process whereby the ripe gamete comes to have the halved or haploid number of the chromosomes.
4. The male and female pronuclei in fertilization are practically equivalent, and possess the same number of chromosomes (overlooking the X and Y chromosomes).
5. In the formation of the ripe spermatozoon no visible part of the chromatinic substance is rejected.

In the cytoplasm of the animal cell it has been shown that two important categories of formed protoplasmic elements exist: namely, mitochondria and Golgi elements. The purpose of this section is to compare and contrast the behaviour of these protoplasmic bodies with the chromosomes of the nucleus. Under the first heading—'That the chromosomes are constant in number in any one species'—we may compare and contrast the Golgi body and mitochondria. While it is not generally possible to gain absolutely explicit evidence by examining the mitochondria in most animals, it is nevertheless true that in some forms the mitochondria are so few and so large that definite counts may be made. As examples I give the following: (*a*) In Paludina the typic spermatid may contain from four to seven spheres. Four is the commonest number. These spheres are subequal in size in those spermatids which contain four spheres and in those which contain seven. (*b*) Wilson (**30**) has shown the same variation to apply in Centrurus, and Retzius (**25**) also in a variety of Molluscs. (*c*) It was shown (**9**) that in Helix aspersa the mitochondria in one spermatocyte

or spermatid were often remarkably different in size and number from those in another example. It is thus clear that the mitochondria are not usually of markedly definite number or size in the germ-cells or somatic cells of any given species. With reference to the Golgi apparatus the same applies. In Helix aspersa (**9**) and in other Molluscs it was shown that the dictyosomes or Golgi bâtonnets could vary in number considerably.

Moreover, examination of preparations of this apparatus in any somatic cells, as well as germ-cells, gives the impression that the Golgi elements are variable to an extreme.

The statement—' In ordinary cell-division each chromosome is halved '—may now be used as a basis for comparison and contrast with what occurs in the mitochondria and Golgi apparatus. In many cases it is difficult to get quite complete evidence as to whether a mitochondrium does divide during cell-division, but the general impression one gathers after examining cells in division is that the mitochondria are sorted out whole and haphazardly. In special cases, e.g. Centrurus (**30**) and Paludina, it is possible that the elongate mitochondria are halved transversely but not longitudinally. In by far the majority of animals it seems tolerably clear that the process of chondriokinesis or distribution of the mitochondria (or chondriosomes) between the daughter-cells is haphazard, and not in any way comparable to the process of karyokinesis. This result has been arrived at by a number of independent workers, and may be taken as established.

The Golgi body in the dividing cell consists of rods or granules (dictyosomes); in most cases these dictyosomes keep around the zone of the amphiaster, often stuck on the asters themselves, and, as with the mitochondria, the observer is impressed with the fact that the whole train of events in dictyokinesis, or the distribution of the dictyosomes between the daughter-cells, is extremely haphazard and much less precise than with the process of karyokinesis. That this is so can easily be shown to be the case in the molluscan germ-cell: in the spermatid of Limax maximus the Golgi apparatus generally

consists of two dictyosomes ('Nebenkern' bâtonnets); but in other cases there may be three, and one never finds a spermatid with a single bâtonnet. It is therefore certain that during dictyokinesis the Golgi elements are not always sorted out equally. That the Golgi rod is divided or halved like the chromosome is unlikely from this evidence, described in detail elsewhere (**9** *a*): in Limax agrestis the spermatocyte has a Golgi apparatus formed of some eight dictyosomes or bâtonnets. This cell divides twice to give rise to four spermatids, but each of the latter only contains two of the Golgi bâtonnets; this shows that in dictyokinesis the bâtonnet is not divided like the chromosome.[1]

With regard to the fact of the maturation of the germ-cells and the reduction of the chromosome number, nothing comparable can be found in either mitochondria or Golgi elements of germ-cells. In the egg the polar bodies rarely contain mitochondrial granules or Golgi elements, and never in such quantity as to suggest a special reduction in number. In the case of the male germ-cells the same applies: the first and second maturation divisions (chondriokinesis) in the male are of the same type, and while they bring about a halving, and then a rough quartering of the original number of mitochondria in the spermatocyte, this process is not of the same nature as the reduction of the chromosomes. The same remark applies to the Golgi elements in dictyokinesis of the male germ-cells during maturation.[1]

In the last stages of gametogenesis in the male no chromosomes are lost: the case of the mitochondria and Golgi apparatus is instructive, for in many Mollusca it has been shown that possibly all the Golgi elements, and much of the mitochondrial matter, are lost during spermateleosis, being sloughed off the tail of the sperm (**9**, **9** *a*). Such seems to occur with the mitochondria in Mammalia; Regaud shows that the head of sloughed off protoplasm of the sperm of rats may contain mitochondria (**24**), though the main bulk of the granules forms part of the sperm. In other words, the chromatin of the

[1] See also Ludford and Gatenby, 'Proc. Roy. Soc.', vol. 92, 1921.

sperm is the only part which is meticulously guarded during spermatogenesis of all animals.

That the male and female pronuclei contain the same number of chromosomes (leaving out the special X or Y chromosomes) is a notorious fact. The sperm never contains as many mitochondrial granules as the egg, and in only one case (*Ascaris megalocephala* (**32**)) has it been shown that at the time of fusion of the ♂ and ♀ pronuclei, the number of mitochondria of the ♂ gamete are about the same as those of the ♀. The above comparisons show conclusively that of all the cell elements the chromosome is the only one whose behaviour is precise and coincident with the expected conduct of bodies directly engaged in the processes of heredity, the results of which, as breeding experiments show, are often of previously calculable exactitude.

As direct bearers of any important or precise factors of heredity, the Golgi body and mitochondria appear to be ruled out by their inexact and variable behaviour in the germ-cell cycle. The chromosomes, and the chromosomes alone, fulfil the necessary conditions.

11. Summary.

Spermatogenesis.

1. The spermatogonium is of the usual type, containing both mitochondria and Golgi apparatus (Pl. 1, fig. 7).

2. The spermatocyte contains the same inclusions as the spermatogonium, but in addition there is to be found, in a large number of cases, a group of granules generally lying near the Golgi elements and giving the microchemical reactions of true yolk, i. e. turning greenish yellow in chrome-osmium fixatives, not staining in haematoxylin or acid fuchsin, and generally dissolved out by strong lipoid solvents (Pl. 1, fig. 1, Y).

3. Nurse-cells often accompany groups of spermatogonia. The nurse-cells contain large quantities of yellowish yolk, as well as fuchsinophil bodies, possibly mitochondrial in nature (Pl. 1, fig. 1, YC).

4. In the spermatocyte there is another group of granules to be found, especially in Kopsch (OsO_4) preparations. These are individually much larger than the four members of the group of yolk-granules, and are about ten to sixteen in number; they go brownish in OsO_4. These larger granules have been traced into the spermatid, where about three or four are present, and appear to form the sides of the mitochondrial part of the sperm-tail (Pl. 1, fig. 2, x, and Pl. 2, figs. 11 and 12, x).

5. During the spermatocyte division prophases, the group of yolk-granules is found to take up a position near the equator of the spindle (Pl. 1, fig. 4, y), and subsequently becomes divided into two smaller groups in later stages of the division (Pl. 1, fig. 5). This process occurs in both maturation divisions, so that each spermatid contains about one-quarter of the yolk-granules of the spermatid. The mitochondria, as is generally the case during cell-division, become altered in such a way that they form a tangled mass of thread-like bodies, which are subequally sorted out into two portions, one in each daughter-cell (Pl. 1, figs. 4 and 5). The larger group of granules which were thought to form part of the tail-sheath were not found during mitosis.

6. The newly-formed spermatid contains the usual inclusions plus the group of yolk-granules (y in Pl. 2, fig. 8). At this stage the sperm-sheath granules are occasionally found, and occur much more often in later stages (Pl. 2, fig. 9, x).

7. The spermateleosis stages, or metamorphosis of spermatid into spermatozoon, are remarkable for the manner of formation of the tail-sheath. The mitochondria become grouped behind the nucleus and around the outgrowing axial filament, while the Golgi elements and yolk-granules take up a position behind the mitochondria (Pl. 2, fig. 9). Tail-sheath granules are usually found in the vicinity (x in Pl. 2, fig. 9).

8. The mitochondria, hitherto single and all approximately equal in size, now begin to run together, like rain-drops, forming groups of larger and smaller granules (Pl. 2, fig. 10, mm and m). This process goes on till only three large subequal spheres are left (figs. 11, 12, and 14), and then these spheres begin to elongate

to form the mitochondrial tail-sheath (Pl. 2, fig. 13 and 16). The tail-sheath granules are seen at x in Pl. 2, figs. 11, 12, and 14.

9. During these stages the Golgi elements tend to become thrown downwards along the length of the sperm (Pl. 2, fig. 16), and this also occurs with the yolk-granules. In a bunch of ripe sperms within the body of the male Saccocirrus small granules are always found on the lower region of the sperm-tails, and there seems to be good evidence that such elements are derived from the Golgi apparatus (Pl. 2, figs. 17 and 18). If the receptaculum seminis of the female is examined, such granules are also found on the tails of the sperms (Pl. 2, fig. 18, GAX).

Fertilization.

10. Saccocirrus is an example of precocious entry of the spermatozoon into the unripe oocyte (Pl. 3, fig. 22). The nuclear head of the sperm alone enters the egg completely at first, while the tail remains plastered on the surface of the young oocyte (Pl. 3, fig. 22, head at SP, fragments of tail at SPT). It is very difficult therefore to say whether these sperm-tail fragments are or are not inside the oocyte cytoplasm at this period. Later on, however, it is quite easily observed that the elements of the sperm-tail do enter the egg, break up further, and form large numbers of spherical granules (consecutive stages given in Pl. 3, figs. 21, SPT, 22, SPT, 24, and 25).

11. In many cases one cannot help believing that these beads, derived from the remains of the sperm-tail, grow in number and in size (cf. Pl. 3, figs. 23 and 25).

12. These beads always remain in the periphery of the egg, but do not seem to take any noticeable part either in the formation of yolk or in any process of fertilization. Careful examination of the periphery of many oocytes reveals the fact that the granules are of two types, one going yellowish in OsO_4, the other going black, as shown in Pl. 3, fig. 21. It was thought that the black granules might have something to do with the black granules noted on Pl. 2, fig. 18, GAX, which were considered to be derived from the Golgi apparatus.

13. The peripheral granules of both types later disappear or become hidden by the formation of clouds of nucleolar deutoplasm.

Oogenesis.

14. By staining, fixing, and by centrifuge experiments it can be shown that the full-grown oocyte of Saccocirrus contains four distinct kinds of formed ' yolk '-granules, i.e. Golgi elements, mitochondria, true yolk, nucleolar extrusions or nucleolar deutoplasm. (See their fixing and staining reactions in a table on p. 18.)

15. The most numerous and chemically most resistant granules are neither mitochondria nor Golgi elements, but are derived from nucleolar extrusions. At a very early stage the oocyte nucleolus buds off pieces which pass through the nuclear membrane, but at first remain stuck on its outer surface (stages in Pl. 3, figs. 23, 24, and 25, NL). At one stage these nucleolar derivatives form an extraordinary picture, being stuck all over the nucleus in the form of pyramidal bodies, whose base adheres to the nuclear membrane (Pl. 3, fig. 23). The nucleolar derivatives stain intensely in haematoxylin or fuchsin (fig. 23), but only go yellowish in osmic acid (fig. 21).

16. Later on these pyramidal granules lose their connexions with the nuclear membrane, but, remaining quite near, become the centres of numbers of large vacuoles which appear (Pl. 4, fig. 29). Inside these vacuoles the nucleolar granules partially break up, and subsequently, after the absorption of the vacuoles, the granules move out further from the nuclear membrane and form clouds of granules in the egg cytoplasm (Pl. 4, figs. 30 and 34). The marked vacuolar stage in the history of the egg seems to occur with suddenness, and is not discoverable at this period in all eggs of this size (Pl. 4, fig. 29). It is just about this stage that great activity is noticeable around the periphery of the nucleus, as shown in Pl. 4, fig. 35, by formol-silver nitrate technique. The nucleolar deutoplasm forms dense clouds of heavy granules throughout the entire egg cytoplasm.

17. If the ovary of Saccocirrus be prepared by a silver

nitrate or osmic acid Golgi-body method, an appearance such as shown in Pl. 3, fig. 19, is seen. Large numbers of crescent-shaped bodies, such as were noted in the spermatocyte (Pl. 1, figs. 6 and 7), occur throughout the cytoplasm. In younger oocytes the bodies of the Golgi apparatus are densely packed and placed to one side of the cell (Pl. 3, fig. 20, GA). Such Golgi elements eventually divide rapidly, and spread out, as fine crescents or slightly elongated rods, through the cytoplasm of the full-grown oocyte (Pl. 4, fig. 32, GA).

18. If the ovary be treated by a chrome-osmium method, and stained in iron alum haematoxylin or acid fuchsin, fine mitochondria become visible (Pl. 3, figs. 23, 24, 25, M). Such mitochondria are difficult and sometimes impossible to see in the youngest oocytes and the oogonia.

19. In chrome-osmium preparations there are also to be seen fine true yolk-spheres, characterized by the fact that they go yellow-green in the fixative and do not stain in haematoxylin or fuchsin.

20. By centrifuging the oocyte, three layers appear, viz. an upper layer formed of true yolk (greenish), a middle clear protoplasm layer, and a lower layer mainly formed of nucleolar deutoplasm, with a mixture of mitochondria.

21. In many oocytes an enigmatic body, much like a secondary nucleus, was noted (Pl. 3, fig. 23, XX, and Pl. 4, fig. 30, XX).

22. The oogonial cytoplasm is oxyphil, and during oogenesis becomes basophil, and then again oxyphil in the full-grown oocyte (p. 16).

Intermediate Cells.

23. In Pl. 1, fig. 3, is a cell found in a male Saccocirrus, and it shows characters intermediate between an egg and a spermatocyte (p. 20).

Discussion.

24. The above facts are discussed on p. 21, and also the probable part played by mitochondria and Golgi bodies in heredity (p. 33).

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13. EXPLANATION OF PLATES 1–4.

Illustrating Professor J. Brontë Gatenby's Paper on the Gametogenesis of Saccocirrus.

Explanation of Lettering.

AS, acrosome. AR, archoplasm or centrosphere. CH, chromosomes. CHO, vitelline membrane. CL, cortical lamellae of egg. GA, Golgi apparatus ('Nebenkern'), Golgi body or element. GAX, body supposed to be a part of the Golgi apparatus. GX, body believed to be forming the acrosome. M, mitochondria. MM, macromitosome or forming middle-piece (mitochondria) of sperm-tail. N, nucleus. NL, nucleolus, or fragments of latter forming nucleolar deutoplasm. NLX, nucleolar bodies homologous with the true nucleolar deutoplasm of egg. NLV, vacuoles around the nucleolar extrusions. SB, spindle-bridge. SPT, sperm-tail. SPZ, spermatozoa. SP, sperm inside young oocyte. V, vacuoles in ground protoplasm of oocyte. X, bodies believed to form a part of the skeleton of the sperm-tail, on each side of the macromitosomal (mitochondrial) spheres. XN, nuclear-like bodies sometimes found in oocytes, possibly secondary nuclei. XY, bodies near asters, possibly part of the Golgi apparatus. Y, yolk-spheres. YC, yolk-cell or nurse-cell.

Scale of Figures.—On Pl. 1, all figures, except number 6, are drawn to the scale indicated in the middle of the plate. The scale for fig. 6 is near the drawing.

On Pl. 2, all figures, excepting 17 and 18, are drawn to the scale in the middle of the plate, the scale for figs. 17 and 18 being near by.

All figures on Pl. 3 are drawn to the scale on the right-hand side. On Pl. 4, all figures, excepting number 34, are drawn to the scale given below fig. 29.

Techniques Used.—M.K., Mann-Kopsch osmium tetroxide method. CH.K., Champy-Kull chrome-osmium acid fuchsin toluidin blue and aurantia method. D.F., Da Fano's cobalt nitrate formol-silver nitrate method.

Plate 1.

Fig. 1.—Full-grown spermatocyte and part of nurse- or yolk-cell on left. In the spermatocyte the Golgi apparatus (GA), the mitochondria (M), and the group of yolk-spheres (Y) are to be seen. The nurse-cell contains

large yolk-spheres (Y) and smaller fuchsinophile bodies, possibly mitochondrial in nature (M). CH.K.

Fig. 2.—Younger spermatocyte from a Mann-Kopsch preparation, showing at X a number (about two) of largish spheres, believed to be identical with the same bodies marked X in figs. 9, 10, and 11, and which seem to take some part in the formation of the tail skeleton of the sperm. At Y are the yolk-granules, and at GA the Golgi apparatus; compare this with fig. 1, in which the apparatus is formed of delicate slightly-curved rods. In fig. 2 the rods are heavily impregnated with OsO_4, and possibly owing to a shrinkage of the centrosphere, they have become much more curved. The mitochondria do not show. M.K.

Fig. 3.—Cell of the spermatocyte series but showing a modification; the mitochondria are small, like those of the egg (fig. 24), and peri-nuclear bodies are present at NLX (compare with egg in fig. 23). At Y is a group of yolk-spheres. CH.K.

Fig. 4.—Metaphase of second spermatocyte division. Note alteration in shape of mitochondria (M), which from their previous granular structure (fig. 1) have become filiform. At Y the group of yolk-spheres has become grouped near the spindle preparatory to being sorted out into two groups as in the next figure. At XY are bodies supposed to be Golgi elements stuck on the poles of the asters.

Fig. 5.—Telophase of second spermatocyte division, the equatorial plate, is forming, and the mitochondria, still filamentous, are grouped near in a special manner, being most numerous near the forming cell-wall. At Y are the yolk-spheres, now sorted out into two groups. CH.K.

Fig. 6.—Spermatocyte, for comparison with the oocytes in fig. 20. M.K. Scale above.

Fig. 7.—Spermatogonium drawn to same scale as spermatocyte in fig. 1. Shows Golgi apparatus consisting of from eight to ten dictyosomes or rods, a spindle-bridge at SB, and the mitochondria at M, grouped near the centrosphere. M.K., counter-stained in Altmann.

Plate 2.

Fig. 8.—Newly-formed spermatid, showing the Golgi apparatus somewhat scattered on the right and the mitochondria surrounding the nucleus. The yolk-granules form a compact group at Y. The centrosome would be on the right side of the nucleus. CH.K.

Fig. 9.—Later spermatid after the outgrowth of the axial filament. The spheres at X are probably of the same nature as those drawn in Pl. 1, fig. 2. The mitochondria have now become grouped behind the nucleus at M, while the Golgi elements (GA) and yolk-spheres (Y) have drifted to the bottom of the elongating cell. The cytoplasm, as in many Kopsch (OsO_4) preparations, is coarsely fibrillar. At GX is a body believed to be forming the acrosome. M.K., counter-stained in Altmann.

Fig. 10.—Later stage. The mitochondria have begun to run together to form a number of larger spheres (MM). At X is one of the large granules seen in fig. 9 and in Pl. 1, fig. 2, while at GX is the same body mentioned in the description of fig. 9. The yolk-granules (Y) form a fine group to one side of the cell. The nucleus in this cell is still spherical, in this being less advanced than that of fig. 9, which is depressed. CH.K.

Fig. 11.—Later spermatid, nucleus now depressed on one side, or cap-shaped. The macromitosomal spheres (MM), 'Nebenkern' of some authors, are larger, not all the same size, and there is still a collection of unused mitochondria at M. The acrosome is seen as a thickened edge of the nucleus at AS. Other parts as before, except that notice should be taken of the fact that the Golgi elements (GA) have become drawn up below the macromitosomal spheres. CH.K.

Fig. 12.—Later stage, all mitochondria have run into the macromitosomal spheres (MM), only two of which are shown. Golgi apparatus still drawn up below the mitochondrial spheres. The cytoplasm is stringy as is often found in osmic-acid preparations. Nucleus further depressed and shrinking in size. M.K., counter-stained in Altmann.

Fig. 13.—Forming spermatozoon, showing elongated macromitosome (MM) and other cell inclusions. CH.K.

Fig. 14.—Macromitosome or mitochondrial spheres, at the stage of fig. 12, but viewed from below. Note skeletal granules at X, and unequal size of spheres. CH.K.

Fig. 15.—Part of tails of fully-formed sperms, at a higher magnification than in fig. 17, to show the bodies marked GAX, which are thought to be Golgi elements. M.K.

Fig. 16.—Forming spermatozoon, at a stage later than that drawn in fig. 13.

Fig. 17.—Bundle of ripe sperms from coelom of male; refer to fig. 15. M.K.

Fig. 18.—Receptaculum seminis of female, to show the presence of the special granules (GAX) on the tails of the spermatozoa, SPZ. M.K.

PLATE 3.

Oogenesis of Saccocirrus.

Figs. 19 and 20.—Four oocytes prepared by the Mann-Kopsch-Altmann method to show Golgi apparatus. The peculiar peripheral granules derived from the sperm-tail (SPT) are shown well. At SP is the head of the spermatozoon, and at NL a peri-nuclear thickening marking partly the nucleolar extrusion, and also as well the peri-nuclear activity, which seems to be something apart from nucleolar extrusion (note also Pl. 4, fig. 35).

Fig. 21.—A later stage showing Golgi apparatus and advanced nucleolar extrusion, NL. The peripheral granules in such Kopsch preparations appear to be of two sorts—those staining quite black, and those yellowish.

Fig. 22 (on the left-hand bottom side of the plate).—Shows two young oocytes, just after entry of spermatozoon (SP). The mitochondria are at M, nucleolar extrusion is just beginning, while the remains of the sperm-tail (SPT) are seen lying, some inside, some outside the oocytes. CH.K.

Fig. 23.—Older oocyte, showing remarkable appearance of nucleolar extrusion (NL), the mitochondria (M), and the sperm-tail remains, which appear to have grown to form numerous spheres around the periphery of the oocyte, at SPT. Other vacuoles (V) are to be seen, which may be the negative image of the non-impregnated Golgi apparatus. At XN is a body thought to be a secondary nucleus of some kind. CH.K. and iron haematoxylin.

Figs. 24 and 25.—Stages earlier than previous figure. At AR is a centrosphere or archoplasm ; in fig. 25 the sperm-tail remains are just passing into the cytoplasm, while in the other figure they have begun to break up into spheres. CH.K.

Fig. 26.—Part of egg cytoplasm, in half-grown egg, showing three categories of granules, true yolk at Y, nucleolar deutoplasm at NL, and the fine mitochondria at M. CH.K. and iron haematoxylin.

Plate 4.

Fig. 27.—Nearly full-grown egg, showing structure of periphery. At CHO is an outer egg-membrane (vitelline membrane of some authors), and at CL are peculiar columns, the cortical lamellae of Lillie. The small granules are mitochondria (stained violet) ; the large, nucleolar deutoplasm (stained yellow). CH.K. fixation followed by Benda's stain.

Figs. 28 and 29. Two oocytes from a Mann-Kopsch preparation, to show the remarkable change which comes over the oocyte at the period of nucleolar extrusion (fig. 29).

Fig. 30.—Young oocyte, after period of main nucleolar extrusion, showing the yolk-granules black and the nucleolar deutoplasm yellowish. At XN is another case of ' secondary nuclei ' ; compare also fig. 23. Taken from a *Saccocirrus papillocercus* (Naples) which had been immersed for six weeks in formol-Flemming.

Figs. 31 and 32.—Mann-Kopsch preparation of nearly ripe egg. Fig. 31 shows appearance of cytoplasm before soaking sections in turpentine, and fig. 32, after treatment for about three hours in turpentine. In fig. 32 the Golgi elements alone resist decolorization.

Fig. 33.—Mann-Kopsch preparation, decolorized in turpentine, to show spreading out of the Golgi apparatus. On the left of the figure is another example of the breaking up of the apparatus ; compare also fig. 28.

Fig. 34.—Nearly ripe egg fixed by Champy-Kull and stained in Benda. Mitochondria were violet, nucleolar deutoplasm yellow-brown.

Fig. 35.—Da Fano preparation to show peri-nuclear activity. Cobalt nitrate-formalin followed by silver-nitrate reduction.